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ANTIDIABETIC EFFECT OF HERBAL AND PHARMACEUTICAL INTERVENTIONS ON ALBINO MICE

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ABSTRACT

Diabetes mellitus is a metabolic disease due to the malfunctioning of B cells of the pancreas. For a very long time, various herbal remedies have been utilized to prevent diabetes. To compare the hypoglycemic impact of an allopathic medication called Metformin, which has a dosage of 650mg/kg BW, with the hypoglycemic effect of a herbal extract (a 2:1 combination of cinnamon and fenugreek) at 200mg/kg BW, this study was designed. Fenugreek and cinnamon contain bioactive ingredients called procyanidin and 4-hydroxy isoleucine, which have anti-diabetic properties. There were four groups of mice. The first group received no treatment; the second group received no treatment; a third group received herbal extract treatment; and the fourth group received metformin treatment. Swiss Albino received a single intraperitoneal injection of alloxan monohydrate at 200mg/kg BW. Group III (the treated group) received 200mg/kg of herbal extract for 14 days. Additionally, treated group IV received metformin for 14 days at a 650mg/kg dose. After treating groups III and IV with an herbal extract and metformin, the bilirubin level gradually and dramatically ($P<0.05$) decreased from the elevated level in the +ve control group. Similarly, serum levels of alkaline phosphate (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) increased dramatically ($P<0.05$) in alloxan-induced diabetic mice due to liver injury. Their level was dramatically ($P<0.05$) reduced after the extract and metformin were administered, coming very near to the -ve control group. This decrease demonstrated the potency of metformin and herbal extract. However, metformin worked better than herbal extract. The liver portion displayed a considerable degree of damage in the diabetic group. Following treatment, the liver section's typical architecture was noted. The trial results indicate that the herbal extract has hypoglycemic properties but to a lesser extent than the allopathic medication metformin.

***Keywords*:** Diabetes Mellitus, Albino mice, Alloxan, Cinnamon and Fenugreek, Histopathology

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1. INTRODUCTION

Diabetes is a metabolic disorder and illness defined by excessive blood glucose levels caused by insufficient insulin production or insulin absorption by cells due to resistance mechanisms. According to the World Health Organization estimate, diabetes will be the leading cause of death and morbidity globally in 2030 (Kaliaperumal et al., 2024). According to the World Health Organization (WHO), diabetes was the leading cause of death in 2019, accounting for roughly 1.5 million deaths. Pakistan is one of the most vulnerable countries to diabetes-related mortality, as the prevalence is higher in poor and middle-income countries. Diabetes affects approximately 463 million persons globally, with type 2 diabetes accounting for 90% of all cases. According to an article by "The News", Pakistan ranks third in the world in diabetes prevalence behind China and India (Azeem et al., 2022). Hyperlipidemia, Obesity, hypertension, physical inertia, expanding age, gestational diabetes history, differences in cultural groups, and genetic factors can add to the acquirer of type II diabetes mellitus (Marinho et al., 2013; Mahmoud et al., 2021). There is no proper treatment for diabetes in medicine. Herbal medications are good alternative supplements for the cure of diabetes (Modglin, 2023). Traditional techniques may provide a natural solution to the diabetic problem (Cheng et al., 2024).

Cinnamon (C*innamomum cassia*) belongs to the family Lauraceae, and many of its members are used as spices (Pages-Rebull et al., 2024). It is well known for being used in the flavoring and beverage industry and is also popular for its medicinal properties (Weeratunge et al., 2024; Li et al., 2024). Cinnamon is currently

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marketed as a therapeutic and preventive agent against many conditions, such as insulin resistance, type 2 diabetes, metabolic syndrome, hyperlipidemia, and arthritis (Mohsin et al., 2023; Tewari et al., 2024). Divergent cinnamon phytochemicals such as cinnamaldehyde, cinnamic acid, and proanthocyanidins (PACs) have been shown. In vivo, bioactivities in cell pathways contribute to better glucose homeostasis (Diana et al., 2012; Yadav et al., 2024).

It has been studied that cinnamon is a characteristic insulin sensitizer and an inhibitor of glycation end product (Peng et al., 2008; Bakhtiar et al., 2024). Cinnamon lowered blood glucose levels, total cholesterol, and triglyceride levels, as shown by research on diabetic mice (Kim et al., 2006). Cinnamon water extracts are more important in lowering blood sugar (Davis and Yokoyama 2011).

Trigonella foenum-graecum is an annual Fabaceae family plant widely known as fenugreek. Fenugreek contains several active chemicals, such as galactomannan, saponins, diosgenin, and 4-hydroxyisoleucine (4-OH-Ile), which have medicinal benefits for people and animals (Rajender et al., 2024). These active chemicals provide fenugreek with various advantages, including anti-inflammatory, anti-carcinogenic, hypoglycemic, antihypertensive, immunomodulatory, and hypocholesterolemic. As a result, fenugreek is considered a beneficial complementary and alternative therapy for managing blood glucose and lipid profile in people with type 2 diabetes and prediabetes (Kim et al., 2023). The issue with using these herbs in treating diabetes, their effectiveness, the method of ingestion, and the prescribed doses are not well documented. The current study aimed to compare the effectiveness of fenugreek and cinnamon together on albino mice with type 2 diabetes to the usual medication metformin.

2. MATERIALS AND METHODS

The study aimed to see how effectively (Cinnamon and fenugreek) combined with Metformin reduce the sugar level in diabetic Albino mice. Metformin was used as a synthetic standard drug.

2.1. Ethical Approval

All experiments, including animal testing, were approved by the ethical committee of GC University Lahore.

2.2. Plant Extract

Cinnamon sticks were taken, washed, and dried before being ground into powder using a mechanical grinder. After making the powder, an aqueous extract of cinnamon was produced by mixing 10g of powder with 200mL of distilled water. The mixture was maintained in a water bath at 88°C for two hours. The concentrate was filtered through a sieve to produce a dilute aqueous extract. This filtrate was stored in a dark bottle and kept at 4°C. Secondly, the fenugreek seeds were taken, cleaned, dried, and used to prepare a powder by crushing them in a mechanical grinder. To prepare an aqueous extract, we added 10g of powder to 250mL of water and kept it in the water bath for 2 hours at 88°C. This was filtered to obtain an aqueous extract using a sieve. This filtrate was held at 4°C and stored in a colored bottle.

2.3. Animals

Albino mice were used as experimental animals. In the experiment, 4-week-old albino mice weighing 25-30g were used. They fed standard food and tap water. Before the trial, the albino mice were maintained in a controlled setting for seven days. The physical environment consists of an average temperature of 25°C, a humidity range of 45–65%, and a breeding duration of 12 hours. At the beginning of the experiment, each mouse was weighed, and then the weight of each mouse was measured weekly during the experiment.

2.4. Induction of Diabetes

Alloxan monohydrate was used to induce diabetes in experimental animals. Since it causes the destruction of islet beta cells in the pancreas. Diabetes was induced in albino mice, according to a study by Vanitha et al. (2014). The albino mice were starved overnight, and a single dose of alloxan monohydrate (250mg/kg BW) was administered. After administration, the mice were given a 10% sugar solution to prevent sudden hypoglycemic conditions. The blood glucose level of mice was measured at 0 (baseline), 7th, 14th, and multi-day. Mice's tails were pierced, and blood was collected using a glucometer to determine blood sugar levels. Diabetic mice were described as having a blood glucose level of 150mg/dL.

2.5. Animal Experimental Models

Albino mice were split into four groups. **Group 1:** Three albino mice with no diabetes induction and no treatment were kept in this group. **Group 2:** Five Albino mice with diabetes were induced with only a single dose of monohydrate alloxan (200mg/kg). The herbal extract and synthetic medicine were given and kept in group 2.

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Group 3: Six diabetic albino mice with treatment were placed in this group. Diabetic albino mice were treated by using herbal extract (Cinnamon and fenugreek) orally (200mg/kg) to group 3 for 21 days (3 weeks). **Group 4:** In this group, six albino mice were placed for treatment. Diabetic albino mice were treated with synthetic (metformin) in group 4 orally (650mg/kg) for 3 weeks.

2.6. Blood Sampling

Albino mice were sacrificed by ketamine as an anesthetic drug. Ketamine was diluted 1:2 in a sterile injection, and intraperitoneal administration of 0.1mL was administered to every mouse. Approximately 1.5mL of blood was taken by heart puncture using 5cc syringes and collected in gel-coated tubes. Blood was permitted to settle for 1-2 hours at room temperature (37°C) for serum separation. Straw-colored supernatant was collected with the help of a pipette and put in the Eppendorf. The remaining part of the blood was spun in a centrifuge at 5000rpm for 15min. After that, the serum was separated in a cylinder and refrigerated at 4°C.

Liver function tests were performed to see the effect on the Liver. Using Rotem's RF Tester Kit, serum was tested for urea, creatinine, and uric acid levels. A microtomy was done to study tissue histology. After histology, the tissues were assessed utilizing a PC-based picture-based examination. The trinocular magnifying tool IRMECO-GmbH with IM-910, 21493 Schwarzenbk/Germany, was shown on a PC via an extension Tek® (Scope picture 3.0).

2.7. Statistical Analysis

The data were presented as mean±SE. The statistical analysis was performed using SPSS version 11.5 software. Analysis of variance (ANOVA) was used, followed by Tukey's multiple comparison test as post hoc was applied for inter-group comparison. In all situations, a $P<0.05$ was considered to be significant.

3. RESULTS

3.1. Blood Glucose Levels and Body Weight

The blood glucose level was recorded in the control group and in all experimentally induced diabetic groups of mice (Table 1). It was found that Alloxan monohydrate (200mg/kg) is the best agent to induce diabetes in Swiss Albino mice. Behavioral and morphological abnormalities were noticed during the trial, including fur thinning, slow body movement, shivering, and timing.

Table 1: Blood sugar level (mg/dL) of mice in different groups

Days	Control	Diabetic	Herbal Treated	Metformin Treated
0	91.33±1.86a	98.4±3.89a	94.5±5.31a	89.50±3.03a
7	81.67±4.41a	160.0±10.37b	169.2±6.64ab	166.8±4.76b
14	93.33±3.33a	164.0±9.14b	173.33±4.77b	141.67±4.77b
21	88.33±4.41a	212.0±12.41b	120.0±10.0ac	98.33±5.426acd

Values (Mean±SE) represent triplicates. Values bearing different alphabets in a row differ significantly ($P<0.05$). Group 1: Albino mice without diabetes - Control. Group 2: Diabetes induced with only a single dose of monohydrate alloxan (200mg/kg). Group 3: Diabetic albino mice treated orally with herbal extract (Cinnamon and fenugreek) (200mg/kg). Group 4: Diabetic albino mice were orally treated with synthetic (metformin) (650mg/kg).

After the diabetes induction in group II (+ve control), a significant decrease in BW of mice was recorded during the experimental period as compared to the control group. This decrease in BW was improved by the treatment with herbal and synthetic drugs (Table 2). During the trial period, group II (+ve control) had a significantly higher blood sugar level than the control group (group I). Diabetic mice treated with 200mg/kg of aqueous extract (cinnamon and fenugreek) and Metformin showed considerable ($P<0.05$) improvement in blood sugar levels compared to the diabetic group of mice (Fig. 1).

Table 2: Body weight (grams) of mice in different groups

Days	Control	Diabetic	Herbal Treated	Metformin Treated
0	26.00±1.00a	27.0±0.89a	28.33±0.21a	29.33±0.61a
7	26.00±0.57a	27.96±0.82a	28.51±0.35ab	30.67±0.42b
14	26.66±0.66a	26.40±0.83a	30.46±0.34b	32.33±0.71b
21	27.06±0.35a	24.42±0.73ac	32.58±0.33bd	35.00±0.86bde

Normal body weight 19.4±1.8g for 5 weeks. Values (Mean±SE) represent triplicates. Values bearing different alphabets in a row differ significantly ($P<0.05$). Group 1: Albino mice without diabetes - Control. Group 2: Diabetes induced with only a single dose of monohydrate alloxan (200mg/kg). Group 3: Diabetic albino mice treated orally with herbal extract (Cinnamon and fenugreek) (200mg/kg). Group 4: Diabetic albino mice were orally treated with synthetic (metformin) (650mg/kg).

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3.2. Effect of Herbal Extract and Metformin on Liver Function Tests in Mice with Diabetic

The bilirubin level increased significantly in group II (+ve control) compared to group I normal mice not treated with Alloxan (Fig. 2). Mice injected with herbal extract (group III) and synthetic medicine metformin (group IV) the reduction in bilirubin level was noticed (Table 3).

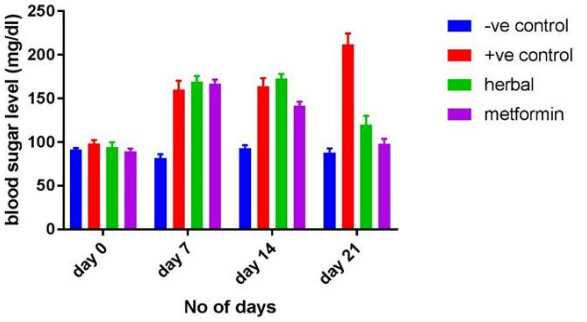

Fig. 1: The effect of herbal extract and metformin on blood sugar level (mg/dL) in control (+ve/−ve) and treated groups.

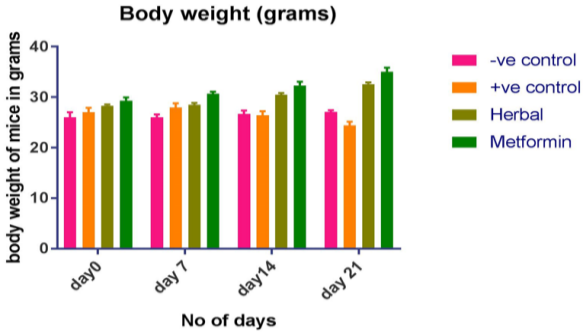

Fig. 2: The impact of natural extract and metformin on the BW (g) of control (+ve/−ve) and treated group.

Table 3: Level of different serum enzymes of liver from 0 day to 21st day

Parameter/Days	Control	Diabetic	Herbal Treated	Metformin Treated
Bilirubin (mg/dL)				
0	0.43±0.033a	0.68±0.086a	0.65±0.085a	0.76±0.056a
21	0.53±0.12a	3.02±0.44bc	1.47±0.17abc	1.70±0.18abc
Alanine aminotransferase (IU)				
0	33.33±3.33a	45.60±5.08a	41.67±3.15a	47.17±5.23a
21	43.33±3.33a	167.00±24.17bc	155.50±10.40bcd	131.67±7.03bcd
Aspartate aminotransferase (IU)				
0	53.33±3.33a	72.00±7.35a	55.33±3.84a	74.83±9.02a
21	56.66±4.41a	170.00±13.04bc	134.33±9.59bde	90.00±2.88adf
Alkaline phosphatase (IU)				
0	68.66±4.66a	71.00±2.30a	68.33±5.05a	65.83±3.65a
21	80.66±3.48a	236.40±6.02bc	155.00±15.43bde	104.50±7.61adf

Values (Mean±SE) represent triplicates. Values bearing different alphabets in a row differ significantly (P<0.05). Group 1: Albino mice without diabetes - Control. Group 2: Diabetes induced with only a single dose of monohydrate alloxan (200mg/kg). Group 3: Diabetic albino mice treated orally with herbal extract (Cinnamon and fenugreek) (200mg/kg). Group 4: Diabetic albino mice were orally treated with synthetic (metformin) (650mg/kg).

Alanine aminotransferase (ALT) level and AST were increased from 45.60±5.08 to 167.00±24.17U/L and 72.00±7.35 to 170.00±13.04U/L respectively, in group II (+ve control) than group I (-ve control) during experimental time duration (Fig. 3 and 4). The dose of Herbal and Metformin, which was given to groups III and IV, indicated a decrease in ALT and AST levels compared to the +ve control group (Table 3). After the induction of diabetes, the diabetic control group had greater levels of alkaline phosphate (ALP) than the normal control group (Fig. 5). Diabetic mice treated with dose of Herbal and Metformin indicated that there was significant (P<0.05) improvement in ALP enzyme too diabetic mice (+ve control) at 21 day (Table 3; Fig. 6).

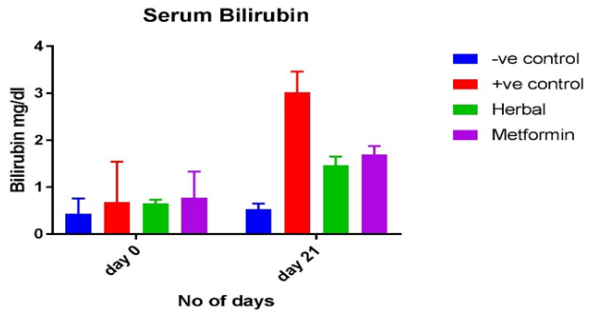

Fig. 3: The effect of Herbal Extract and Metformin on the Bilirubin (mg/dL) level in the control (+ve/-ve) and treated groups.

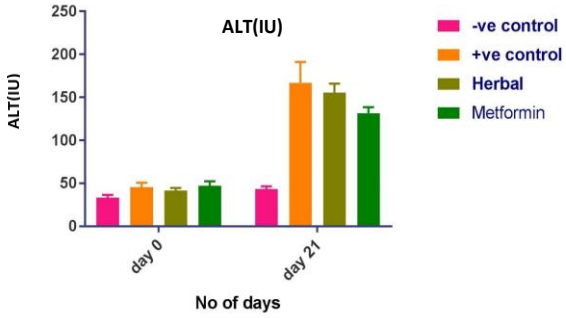

Fig. 4: The effect of Herbal Extract and Metformin on the ALT (IU) level in the control (+ve/-ve) and treated groups.

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3.3. Histology

We performed histology of the liver of all mice. After that, we noticed the typical arrangement of hepatocytes without lesions and standard sinusoidal space structure in the negative control group (un-diabetic). While a person with diabetes, a high degree of liver damage with a dilated central vein and large sinusoidal spaces were observed in a small portion of mice's liver. After herbal and synthetic (metformin) treatments, groups 3 and 4 showed promising results (Fig. 7). A minor degree of liver damage was seen in the treated group, and normalization of hepatocyte cells was seen in the fourth (metformin-treated) group (Fig. 7).

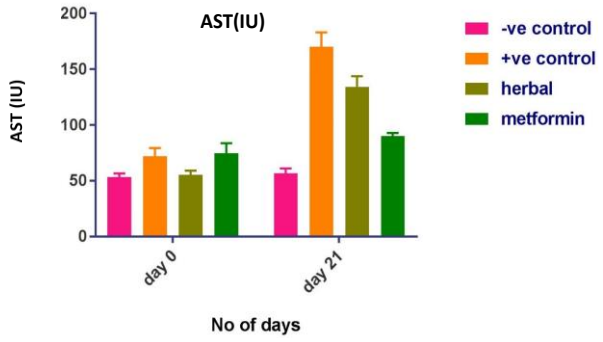

Fig. 5: Effect of herbal extract and metformin on AST (IU) level in control (+ve/-ve) and treated group

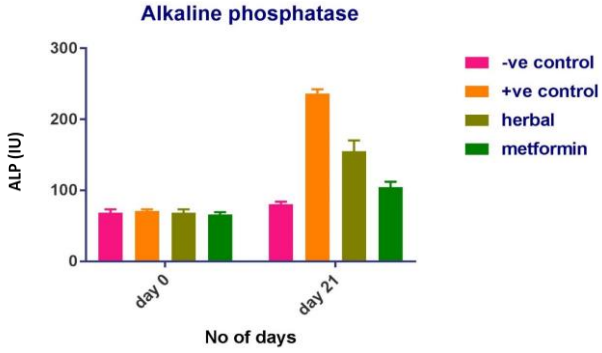

Fig. 6: The effect of Herbal Extract and Metformin on the ALP (IU) level in the control (+ve/-ve) and treated groups.

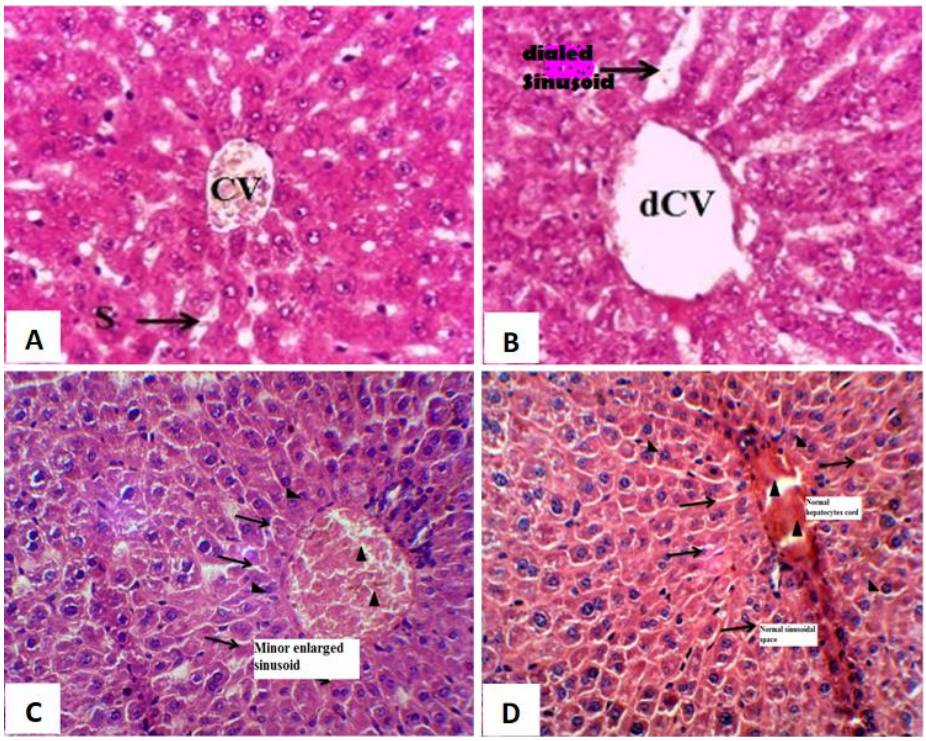

Fig. 7: Photomicrograph of liver of A) Negative control (non-diabetic) CV (central vein), B) Positive control (Diabetic) dCV (dilated central vein), C) Herbal treated (Cinnamon and Fenugreek in combination) and D) Synthetic (Metformin treated). Hematoxylin and eosin staining. Magnification = 40X.

4. DISCUSSION

Herbal medications are produced from the leaves, roots, seeds, bark, fruit, stems, or flowers of various plants that have medical properties or are thought to have therapeutic advantages (Amir et al., 2023). Traditional medicine obtained from medicinal plants greatly potentiates the innovation of new antidiabetic drugs (Jung et al., 2006).

In the present study, diabetes was formed in Albino mice. Alloxan monohydrate causes diabetes mellitus by producing glucose intolerance and hyperglycemia because of lower insulin concentration levels or faulty insulin activity (Sicree et al., 2006). The mixture of Cinnamon and Fenugreek in the ratio of 2:1 was seen to considerably decrease the blood glucose after 3 weeks' treatment in group III. Cinnamon and fenugreek extract used in diabetic albino mice caused substantially reduced blood sugar/glucose levels ($P<0.05$).

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This is due to high glucose transportation or inhibition of glucose absorption in the intestine (Kumar et al., 2011). Many studies showed that mice treated with 650mg/kg of metformin drug (Mukul & Gupta, 2008) showed a significant decrease in blood glucose levels (Mohammed & Wasfi, 2011). Our study demonstrates considerable ($P<0.05$) lowering in mice BW because of increased body metabolic rate and dehydration. The herbal extract dose (200mg/kg) caused a considerable rise in the mice BW compared to another diabetic group (Tiwari et al., 2014).

In group II, the Bilirubin, AST, ALT, and ALP levels increased, but when treated with Herbal Extract and Metformin, the levels decreased compared to the +ve group (II). Improvements in serum Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), and Aspartate Aminotransferase (AST) levels in diabetic mice powerfully recommended damage to liver functions because of induction of diabetes. When diabetes was treated with Herbal Extract, an essential reduction in ALT, AST, and ALP concentration was observed. Histology of the liver was confirmed because of the hepatocyte distortion in diabetes-induced albino mice. However, herbal extract treatment maintained the basic structure of the liver and prohibited its damage like the control group (Motshakeri et al., 2014).

5. CONCLUSION

In conclusion, a mixture of Herbal Extract (Cinnamon and Fenugreek) (200mg/kg) and synthetic medicine (Metformin) (650mg/kg) proved to be an effective anti-hyperglycemic agent at their recommended dose. Moreover, histological examination of the liver also indicated the effectiveness of herbal and synthetic medicine in preventing structural damage and maintaining normal function.

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